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Phage Therapy, A Promising Approach to Combat Antibiotic Resistance

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Abstract: ***Resistance against antibiotics is a worldwide public health concern. For solving this problem, interest in phage therapy has increased. Directly phage viruses can be applied or phage derived proteins like holin, phage lysins can be isolated and purified and then can be applied to treat bacterial diseases. But like any other therapeutic approaches, phage therapy has some disadvantages also. Furthermore, the investigations regarding phage therapy are limited and further clinical trials are required to make this treatment widely available for human use.***

Keywords: Bacteriophage, Lysin, Holin, Antibiotic Resistance, Therapy

1. Introduction

Increasing resistance of bacteria to the traditionally used antibiotics is a serious public health problem that raises concern in the whole world and it is associated with unnecessary use of antibiotics. Due to this , new antimicrobial therapeutics, beside the antibiotics should be developed immediately. Phage therapy can be one of these new strategies through which deadly bacteria can be killed by using the bacteriophages.

All viruses are not harmful to humans. Bacteriophages are the viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial genome and cause the bacterium to burst. More than a hundred years ago phage therapy was discovered which is the use of bacteriophage viruses to treat bacterial infections (D'Hérelle, 1931). In the year of 1896, a British bacteriologist, Ernest Hankin, reported (Hankin,.1896) the occurance of the antibacterial activity against Vibrio cholera which he noticed in the waters of the Ganges and Jumna rivers in India, and he suggested that an unidentified substance was responsible for this phenomenon and for limiting the spread of cholera epidemics. After Two years, Gamaleya, the Russian bacteriologist observed a similar phenomenon when he was working with Bacillus subtilis. Almost ten years before the discovery of penicillin, the practice of phage therapy was developed as a treatment for bacterial infections. Bacteriophages, are bacteria - specific viruses that have been used as a treatment against pathogens such as Shigella dysenteriae as early as 1919. Phages make up the most abundant biological entity on Earth and play an important role in regulating bacterial populations; phages are responsible for the death of approximately 20% - 40% of all marine surface bacteria every 24h (Wittebole X, 2014).

In contrast to antibiotics, phage therapy specifically lyses the host bacteria and does not affect non - host bacteria. The phage abundance is in proportion to that of the host pathogenic bacteria, so, as soon as the inactivation of the host pathogenic bacteria, when the host bacteria diminish, the phage count also decreases, which maintains the microbial stability and diversity.

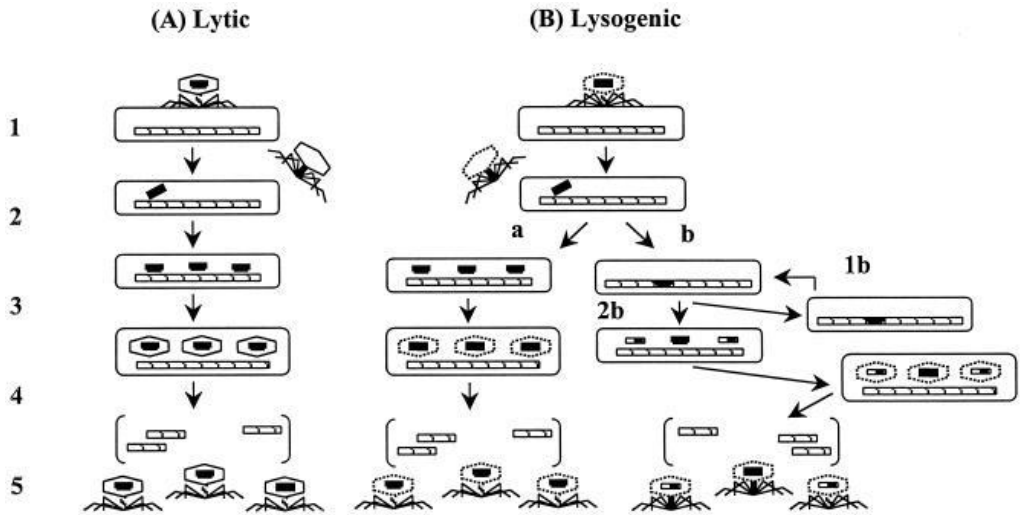
Main aim of this review is discussing the use of phage therapy and progression of current research on the feasibility of phage - based infection control with a focus on antibiotic resistant bacterial infections.

Basic details of Bacteriophage

A) Structure

Different bacteriophages have different structures, complexity, shape and genetic material (Keen, 2015). Phage genome is made up of single or double stranded RNA or DNA. Most of the bacteriophage head is icosahedral in shape and has a tail which is made up of helical symmetry protein (White, 2020). Head consist of 2000 capsomere with nucleic acid enclosed within it. The tail consists of inner hollow tube which is surrounded by a contractile sheath with 24 annular rings. Basal plate is present at the distal portion of the tail and tail fibre, and spicules are attached with it. Basal plate has proteins which can identify and attach with the receptor protein of specific host bacteria (White, 2020).

B) Phage Life Cycle



Replication cycles of lytic and lysogenic phages. (A) Lytic phages: step 1, attachment; step 2, injection of phage DNA into the bacterial host; step 3, shutoff of synthesis of host components, replication of phage DNA, and production of new capsids; step 4, assembly of phages; step 5, release of mature phages (lysis). (B) Lysogenic phages: steps 1 and 2 are similar to those of lytic phages (i. e., attachment and injection, respectively); starting with step 3, lysogenic phages can, among other possibilities, initiate a reproductive cycle similar to that of lytic phages (a) or integrate their DNA into the host bacterium's chromosome (lysogenization) (b). Lysogenized cells can replicate normally for many generations (1b) or at some point undergo lysogenic induction (2b) spontaneously or because of inducing agents such as radiation or carcinogens, during which time the integrated phage DNA is excised from the bacterial chromosome and may pick up fragments of bacterial DNA. (Sulakvelidze, 2001)

2. Application of Phage Therapy

Current research using animal models have discovered that phage therapy against a range of clinically significant pathogens. Oral administration of phage saved 66.7% of mice from death by gut - derived sepsis cused by *P. aeruginosa*, compared to 0% in the control group (Watanabe R, 2007). Only one dose of phage with *C. difficile* administration was sufficient to give prophylaxis against infection in a hamster model of *Clostridium difficile* (*C. difficile*) - induced ileocecitis (Ramesh V, 1999). Studies on animal recealed promising results for multidrug - resistant *E. coli* O25: H4 - ST131 (Pouillot F, 2012), *Vibrio parahaemolyticus* (Jun JW, 2014), *S. aureus* (Soothill JS, 1992) and *A. baumanii*. There is a clear indication that phages are capable of restoring antibiotic sensitivity in drug resistant bacteria, for example in the case of multidrug - resistant *P. aeruginosa* (Chan BK, 2016).

One of the most widely accepted approaches in phage therapy is the isolation and purification of Lytic enzymes, encoded by phages, have functional similarity to the lysozyme which is an eukaryotic enzyme with antimicrobial activity. As soon as the lytic cycle is on, expression of genes encoding for phage lytic enzymes starts within the host bacteria and it helps the phage to hydrolyze the bacterial cell wall for releasing the newly formed phage particles. Mainly two types of proteins are responsible for the the lysis of the host bacterium. They are Holin, a transmembrane protein and endolysin (lysine), which is peptidoglycan cell wall hydrolase. Simultaneous functioning of these two proteins triggers the destruction of the cell wall of bacteria. “Molecular clock” of the phage lytic cycle is holin. When viral assembly starts within the cytoplasm of host bacteria, the protein holin is accumulated in the cell membrane. At the termination of the lytic cycle, holin molecules create a pore on the cytoplasmic side of the cell membrane, which allows the lysin molecules to reach and hydrolyze the bacterial cell wall (Lin DM, 2017)

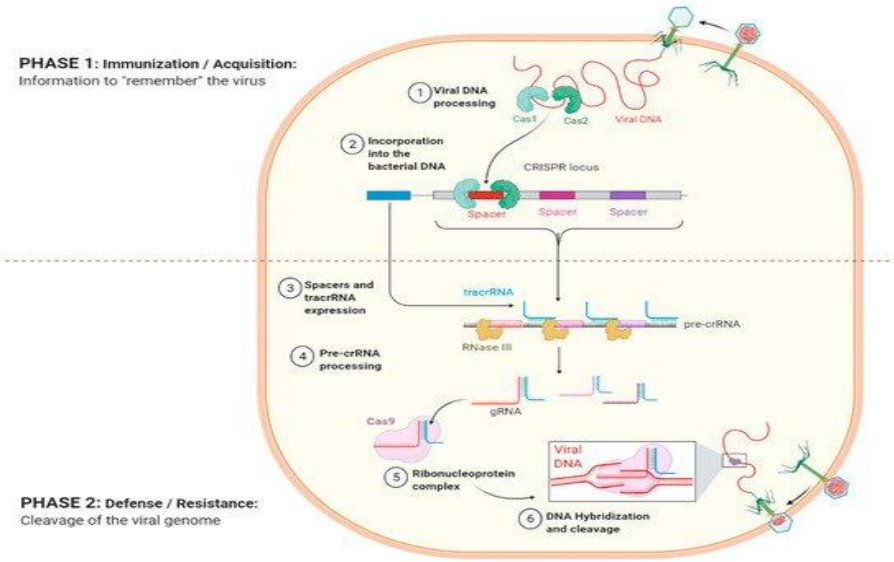
By recombinant DNA technology, large scale mass production of lysin is possible. The gene for phage lysin has been cloned and inserted into E. coli for over expression and purification (Keary R, 2016). lysins cleaves the bacterial cell wall, so they are not much efficient against gram - negative bacteria which have outer membrane made up of lipopolysaccharide. For killing gram negative bacteria by lysin, scientists have started to try to make artificially modified lysine known as Artilysins, which can easily penetrate the outer membrane of gram negative bacteria (Briers Y, 2014). It is believed that bacteria will not evolve resistance against lysins because their target sites are on the peptidoglycan cell wall which is crucial for the viability of the bacterial cell (Roach DR, 2015). Biologically engineered phage lysins would be very easily administrated in comparison to natural preparations of phage, which has short shelf life and they are removed by the reticuloendothelial system of the host. Application of combined therapy of lysins along with antibiotics, is considered to be more efficient than using only antibiotics or only lysins against the drug resistant pathogens such as MRSA (Schuch R, 2014)

Advantages and disadvantages of phage therapy

1) **Specificity of Phages**
   Phages are very specific to the host. If infections with more than one host, it is necessary to use a phage Cocktail.
2) **Bactericidal effects**
   Lytic phages infect host bacteria and cause cell death, compared to certain bacteriostatic antibiotics.

3) **Active on - site propagation**
Phages increase the concentration in the host as they replicate, theoretically requiring only one therapeutic dose.
4) **Lower level of toxicity**
Phages exist in large amounts in the biosphere, it is possible to isolate and purify the phage required to achieve certain bacteria.
5) **Composition and administration versatility**
Various phages can be converted in a cocktail to target several bacteria simultaneously. The type of administration can also vary, liquid, powder, ointment, tablets.
6) **High efficiency against MDR strains**
Since the phage and bacterial resistance mechanisms are different, bacteria resistant to certain antibiotics can be treated with the use of phage therapy.
7) **Efficacy against biofilm**
Phages can penetrate through biofilms. Part of this capacity is due to the presence of depolymerases and lysins
8) **Comparatively lower production cost than antibiotics**
The costs associated with discovering phage isolation and purification are relatively low
9) **Low environmental harm**
Phages are natural components of the environment.

Some of the disadvantages of phage therapy are: (i) Difficulty of treating polymicrobial infection. (ii) Food and Drug Administration (FDA) or the European Medicines Agency (EMA) does not give approval to this therapy due to concerns about phage resistance. (iii) Lack of clear information regarding phage selection criteria (Silva C, 2022) iv) CRISPR - Cas System, is another unique antiviral mechanism that saves the host by degrading foreign DNA. This system acts adaptive immune system of bacteria. When the phage virus enter inside the bacterium US CRISPR - Cas system is activated. This recognizes the foreign DNA and enzymes (Cas) cut this material and insert them into a certain region of the bacterial genome, known as CRISPR locus. During the next infections, bacteria containing the pieces of viral DNA introduced into the CRISPR locus, synthesizes an RNA from this sequence. The Cas enzyme will associate with this RNA and then make its way to the viral DNA, which is then cleaved and thus inactivated (Bondy - Denomy, 2013). Phages have made ways to inactivate CRISPR - Cas systems. Phages can escape CRISPR interference by specific mutations (Le Rhun, 2019).



CRISPR - Cas9 Adapative Immune System of *Pseudomonas aeruginosa* against bacteriophage. After infection, the Cas1 - Cas2 complex identifies the exogenous DNA and integrates a portion of it into the CRISPR array and gives rise to a new spacer. The bacterium inserts a fragment of phage DNA into its genome as a spacer into the CRISPR array, this spacer will work as memory and allows the bacterium to identify the same threat when reinfection occurs. The CRISPR array is transcribed as a long precursor CRISPR RNA (pre - crRNA). The host RNase III recognizes the tracrRNA: crRNA - Cas9 complex and cuts both tracrRNA and crRNA. The Cas9 protein and the gRNA form a ribonucleoprotein complex through interactions between the gRNA scaffold and surface - exposed positively - charged grooves on Cas9. Then Cas9 undergoes a conformational change upon gRNA binding that change this molecule from non - DNA binding inactive conformation into an active DNA - binding conformation (Silva C, 2022).

3. Conclusion

Due to lack of new antibiotics against the resistant strains in the pipeline require the discovery of alternative therapies, such as phage therapy. Many publications, some of which are mentioned in this review, suggest that phage therapy may be effective therapeutic approach in coming future. But many vital points that must be addressed before lytic phages can be widely usd for therapeutic purposes. Phage lysins may be a much more efficient therapeutic molecule for their ease of production, purification, and storage. The increased efficiency of antibacterial agents when used in combination with phage, phage - derived lytic proteins, bioengineered phage will be necessary for combating the growing problem of multi drug resistant bacterial infections.

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